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Authority for the Patent Cooperation Treaty

Applicants: The Rockefeller University et al.
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Dear Sir:

RESPONSE TO WRITTEN OPINION

This is in reply to the Written Opinion mailed 11 May 2005 setting a three (3) month period for response.

Claims 1-6 have been suggested to lack novelty in view of Trempus et al. (2003) J. Invest. Dermatol. 120:501-511. Applicant respectfully disagrees.

Trempus et al. teach the isolation of cells based upon the presence or absence of CD34 and presence or absence of alpha 6 integrin. The CD34+/alpha 6+ population of cells isolated by Trempus et al. encompasses both suprabasal and basal cell populations. In contrast, the instant specification teaches at page 52-53 that the level of expression of alpha 6 integrin (or other selected marker) is essential for the

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isolation of a basal from a suprabasal cell (i.e., cells set forth in claims 3 and 4).

Claims 7 and 9-16 have been suggested to lack novelty in view of Yuan et al. ((2002) *J. Neurosci. Res.* 70:529-545), or Roy et al. ((1999) *J. Neurosci.* 19:9986-9995), or Fujikawa et al. ((2003) *J. Hepatol.* 39:162-170), or Coffin et al. ((1998) *Gene Ther.* 5:718-722). It is suggested that the method by which the cells are produced fails to differentiate the cells isolated thereby from the art. Claims 7-16 have also been suggested to lack novelty as being anticipated by Bartz et al. ((2003) *J. Immunol. Methods* 275:137-148). Applicant respectfully disagrees.

Yuan et al. teach EGFP fused to an oligodendrocyte-specific promoter and isolation of cells expressing the same.

Roy et al. teach hGFP fused to a human early promoter for the oligodendrocytic protein CNP2 and isolation of cells expressing the same.

Fujikawa et al. teach GFP fused to cytomegalovirus enhancer β-actin promoter and isolation of cells expressing the high levels of GFP from cells expressing low levels of GFP.

Coffin et al. teach GFP fused to CMV promoter and isolation of cells expressing the same.

Bartz et al. teach sorting of cells based upon CD34 or CD133 expression and further sorting based upon cutaneous leukocyte antigen.

None of the cited references (Yuan et al., Roy et al., Fujikawa et al., Coffin et al., or Bartz et al.) teach an isolated cell harboring a nucleic acid sequence encoding a regulatable transcription factor operably linked to a promoter which is active in a slow-cycling cell and a nucleic acid sequence encoding a reporter protein operably linked to a

regulated promoter to which the regulatable transcription factor binds, wherein the cell was isolated by activating the regulatable transcription factor so that expression of the reporter protein is increased; inactivating the regulatable transcription factor so that expression of the reporter protein is decreased; incubating the cell for a sufficient amount of time so that the cell goes through one or more cell cycles to generate a population of cells; detecting the amount of reporter in the population of cells; and sorting the population of cells by the amount of reporter present in each cell. Accordingly, cells isolated by the method of the instant invention are distinct from cells isolated in the art, because the cells of the instant invention express a regulatable transcription factor operably linked to a promoter which is active in a slow-cycling cell and a nucleic acid sequence encoding a reporter protein operably linked to a regulated promoter to which the regulatable transcription factor binds. In contrast, the cited references teach cells harboring a nucleic acid sequence encoding a reporter protein operably linked to a promoter which is active in a slow-cycling cell. Further, the "pulse-chase" approach of the instant invention is distinct from the prior art in providing cells with high levels of reporter versus daughter cells which have diluted amounts of reporter (see paragraph bridging pages 18 and 19 of the instant specification). Cells isolated in accordance with the prior art methods contain stems cells as well as daughter cells because all GFP expressing cells are isolated.

Claims 17-18 have been suggested to lack novelty as being anticipated by Punzel et al. It is suggested the Punzel et al. teach the culture and expansion of human hematopoietic stem cells, by growing the cells on fibroblast feeder cells using

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LTBMC medium, a medium containing calcium chloride (0.219 g/L). Applicant respectfully disagrees.

Calcium chloride has a molecular weight of 111.0 g/mol. At 0.219 g/L, the concentration of calcium in the medium of Punzel et al. would be 0.00199 mol/L or 1.99 mM, which is outside the instantly claimed range of 0.2 mM to 0.5 mM. Page 68, lines 1-2, of the specification teach that the level of calcium of Punzel et al. (i.e., 1.5 mM) is used to induce differentiation in combination with reduced serum content.

Further, while both claims 17 and 18 have been suggested to lack novelty in view Punzel et al., this reference is silent to inhibiting cell growth by contacting a cell with BMP6 or FGF-18 as set forth in claim 18.

Claims 19-20 have been suggested to lack novelty as being anticipated by Krestel et al. ((2001) *Nucl. Acid Res.* 29:e39). It is suggested that Krestel et al. teach the generation of transgenic mice using a transgene encoding humanized GFP that is regulated by doxycycline. Expression is activated when the transcription factor tTA was expressed by the transgene. Applicant respectfully disagrees.

Krestel et al. teach humanized GFP fused to a TetO₇/CMV minimal promoter and the tTA transcription factor, which binds TetO₇, fused to the promoter of the α -subunit gene of calcium-calmodulin dependent kinase II. The reference teaches that using doxycycline regulation, GFP expression was observed in neurons of the olfactory system, neocortical, limbic lob, basal ganglia, thalamic, pontine and medullary structures, spinal cord, the eye, and Purkinje cells in the cerebellum. This reference does not however teach a long-lived reporter molecule (e.g., a H2B-GFP fusion protein; see page 25 of the specification) as set forth in claim 19. This reference also fails to teach a tissue-specific promoter fused to the tTA

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transcription factor. Accordingly, in an earnest effort to clarify the instant non-human transgenic animal, claim 20 has been amended. Enclosed is substitute page 75. Support for a tissue-specific promoter can be found at page 19 of the instant specification and the paragraph bridging pages 21-22. No new matter has been added by this amendment.

Reconsideration is respectfully requested.

Respectfully submitted,

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Date: 28 July 2005

JML:sts

Enclosure: substitute page 75

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19. A non-human transgenic animal model whose genome contains a transgene comprising a nucleic acid sequence of a tetracycline-response element operably linked to a nucleic acid sequence of a minimal promoter which is
5 further operably linked to a nucleic acid sequence encoding a long-lived reporter protein.

20. The non-human transgenic animal model of claim 19, wherein said animal model further contains a transgene
10 comprising nucleic acid sequences of a tissue-specific promoter operably linked to a nucleic acid sequence encoding a tetracycline-responsive transcription factor that binds to the tetracycline-response element so that the reporter protein is expressed.